American Journal of Hematology 51:32–36 (1996)

# Two Different Mutations in Codon 97 of the β-Globin Gene Cause Hb Malmö in Sweden

**Britta Landin, Stig Berglund, and Kristina Wallman**

Department of Medical Laboratory Sciences and Technology, Division of Clinical Chemistry, Karolinska Institute, Stockholm (B.L.); Department of Internal Medicine, University of Lund, Malmö General Hospital, Malmö (S.B.); and Department of Internal Medicine, Central Hospital, Falun (K.W.), Sweden

**An abnormal hemoglobin with increased oxygen affinity, Hb Malmö [$\alpha_2\beta_2$97(FG4)His→Gln], was found to cause erythrocytosis in two apparently unrelated Swedish families. Direct nucleotide sequencing of amplified DNA demonstrated a CAC→CAA substitution in one family and a CAC→CAG substitution in the other. Both mutations resulted in a His→Gln substitution in codon 97. This finding prompted us to examine the possible point mutations underlying the different hemoglobin variants reported in the literature.**


**Key words: hemoglobins, abnormal; beta-globin; point mutation**

## INTRODUCTION

Hb Malmö [$\alpha_2\beta_2$97(FG4)His→Gln] is an abnormal hemoglobin causing familial erythrocytosis which was described in 1970 [1] in a family (family A) living in southern Sweden. Soon afterwards it was found in a family in the US which had no obvious relation with the Swedish family, and Hb Malmö was then pointed out as the first erythrocytosis-producing hemoglobinopathy found in two apparently unrelated kindreds [2]. Later Hb Malmö was also found in a Sicilian family where the propositus was a compound heterozygote for Hb Malmö and $\beta^0$-thalassemia [3]. From the proband's appearance (blond hair and blue eyes) a relation to the initial Swedish finding was assumed [3].

After the initial finding in four generations of family A, no additional cases of Hb Malmö were discovered in Sweden until 1993. Then a sample from a young man with erythocytosis was subjected to hemoglobin investigation. Routine electrofocusing demonstrated an abnormal fraction and sequencing of the coding parts of the β-globin gene revealed a substitution CAC→CAG at codon 97, thus corresponding to Hb Malmö. Nucleotide sequencing of amplified material obtained from frozen hemolysates from family A did, however, clearly demonstrate a CAC→CAA substitution. This indicates that in Sweden, Hb Malmö originates from two separate mutations.



## MATERIALS AND METHODS

Hematological data were obtained with local routine methods. Quantification of Hb F [4] and Hb $A_2$ [5] was made by ion-exchange high performance liquid chromatography (HPLC). Electrofocusing was routinely performed according to a slight modification [5] of the method originally published for separating Hb Malmö from Hb A [6].

DNA was extracted [7] from fresh EDTA blood (family B) or from a 23-year-old sample of hemolyzed red cells (family A). The latter was originally obtained by hemolysing washed red blood cells with distilled water, extracting with 0.1 volume carbon tetrachloride and adjusting the water phase to 0.1 M $NaH_2PO_4$, 10 mM KCN, 0.05 mM EDTA pH 7.0 prior to storage at −70°C. Amplification, purification of the amplified material, and nucleotide sequencing followed routine procedures [8] except that 16

Received for publication April 7, 1995; accepted August 9, 1995.

Address reprint requests to Britta Landin, M.D., Ph.D., Department of Clinical Chemistry, Huddinge Hospital C1 62, S-141 86 Huddinge, Sweden.

At time of investigation Dr. Landin was working at the Department of Clinical Chemistry, University of Lund, Malmö General Hospital, Malmö, Sweden.

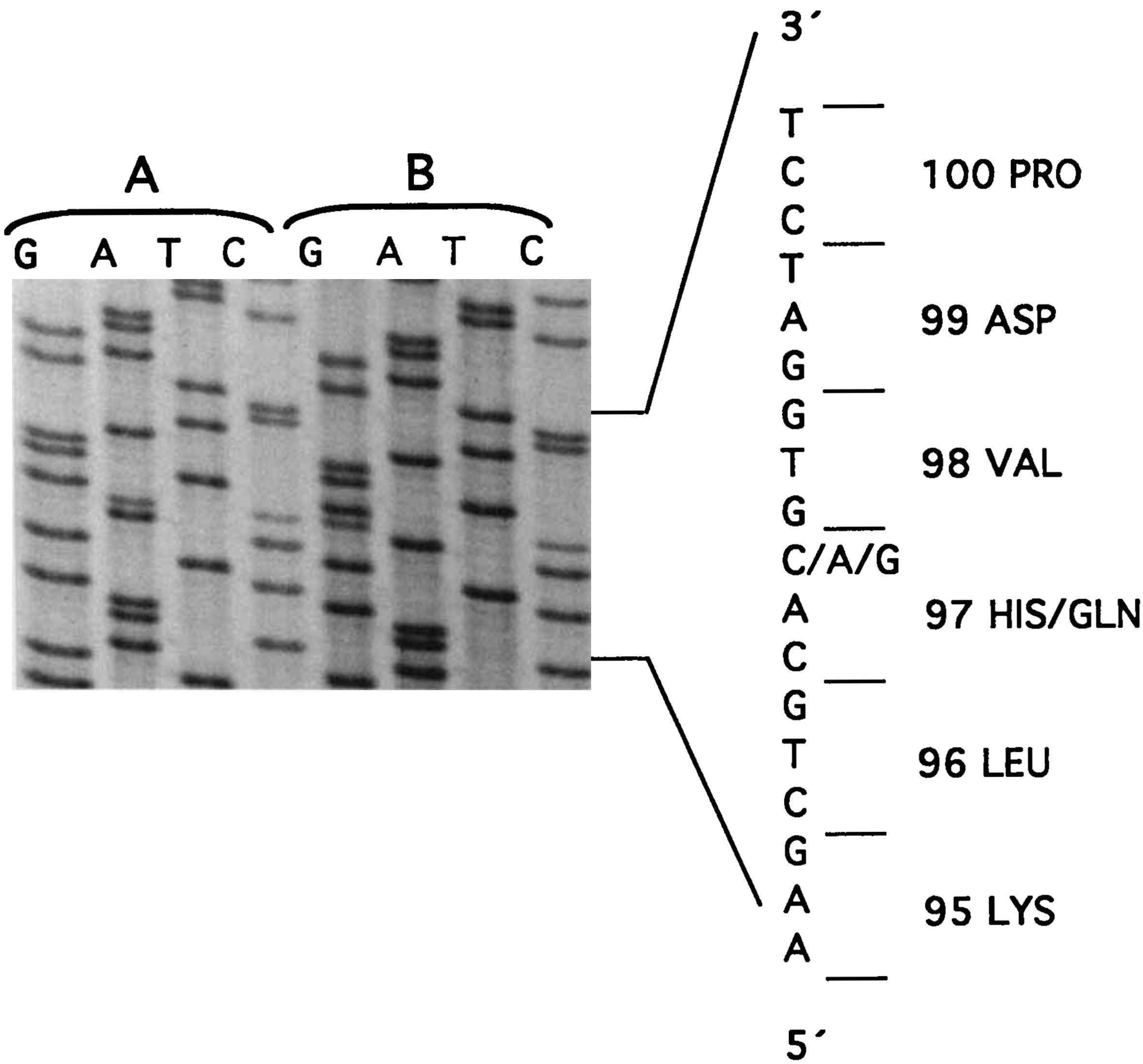


**Fig. 1. Sequence analysis of amplified β-globin DNA from members of two different families, both showing heterozygosity for Hb Malmö. In family A the codon 97 mutation CAC→CAA was found, while in family B the mutation was CAC→CAG. Both mutations will result in a His→Gln substitution. The sequencing primer corresponded to codon 62 to 68.**

μg bovine serum albumin was included in the 100 μL incubations used for amplifying DNA from family A. Six different polymorphic restriction enzyme sites in the β-globin cluster were amplified and cleaved to give an indication of haplotype pattern [9].

## RESULTS

The proband in family B was a 26-year-old male with hemoglobin 200 g/L, hematocrit 59%, WBC 6.4 × $10^9$/L and PLT 186 × $10^9$/L. $HbA_2$ was 2.8% and HbF was not detectable. Electrofocusing demonstrated a distinct abnormal anodal fraction. No major variant fraction was separated at the HPLCs used for $HbA_2$ or HbF quantitations but the HbF chromatogram [4] demonstrated a duplicated $HbA_{1c}$ peak. The fraction eluting somewhat prior to the normal $HbA_{1c}$ fraction from the cation exchange resin contained 1.3% of the total hemoglobin content and probably comprised the N-terminally glycated form of the variant hemoglobin, while the normal $HbA_{1c}$ fraction contained 2.1% of total hemoglobin.

Nucleotide sequencing demonstrated a substitution CAC→CAG at codon 97 in the β-globin gene (Fig. 1), thus corresponding to Hb Malmö. Family B lived 800 km from family A and had no known relatives in the southern part of Sweden where family A lived. Since it was realized that two different nucleotide substitutions might cause the same hemoglobin variant, we also tried

**TABLE I. β-Globin Variants Described in the Literature, Where More Than One Nucleotide Substitution Could Cause the Same Phenotype**

| Normal | | | Variant | | |
|---|---|---|---|---|---|
| Position | Code | Amino acid | Possible codes | Amino acid | Name |
| β2 | CAC | His | CAG/CAA | Gln | Hb Okayama |
| β15 | TGG | Arg | AGG/CGG | Arg | Hb Belfast |
| β17 | AAG | Lys | ATT/AAC | Asn | Hb J-Amiens |
| β19 | AAC | Asn | AAG/AAA | Lys | Hb D-Ouled Rabah |
| β30 | AGG | Arg | AGT/AGC | Ser | Hb Tacoma |
| β37 | TGG | Trp | AGG/CGG | Arg | Hb Rotschild |
| β40 | AGG | Arg | AGT/AGC | Ser | Hb Austin |
| β42 | TTT | Phe | CTT/TTG/TTA | Leu | Hb Louisville |
| β46 | GGG | Gly | AGG/CGG | Arg | Hb Gainesville-GA |
| β57 | AAC | Asn | AAG/AAA | Lys | Hb G-Ferrara |
| β59 | AAG | Lys | AAT/AAC | Asn | Hb J-Lome |
| β60 | GTG | Val | TTG/CTG | Leu | Hb Yatsushiro |
| β61 | AAG | Lys | AAT/AAC | Asn | Hb Hikari |
| β65 | AAG | Lys | AAT/AAC | Asn | Hb J-Sicilia |
| β80 | AAC | Asn | AAG/AAA | Lys | Hb G-Szuhu |
| β82 | AAG | Lys | AAT/AAC | Asn (→Asp) | Hb Providence |
| β90 | GAG | Glu | GAT/GAC | Asp | Hb Pierre-Bénite |
| β92 | CAC | His | CAG/CAA | Gln | Hb St. Etienne |
| β95 | AAG | Lys | AAT/AAC | Asn | Hb Detroit |
| β97 | CAC | His | CAG/CAA | Gln | Hb Malmö |
| β99 | GAT | Asp | GAA/GAG | Glu | Hb Coimbra |
| β101 | GAG | Glu | GAT/GAC | Asp | Hb Potomac |
| β102 | AAC | Asn | AAG/AAA | Lys | Hb Richmond |
| β103 | TTC | Phe | CTC/TTG/TTA | Leu | Hb Heathrow |
| β104 | AGG | Arg | AGT/AGC | Ser | Hb Camperdown |
| β108 | AAC | Asn | AAG/AAA | Lys | Hb Presbyterian |
| β109 | GTG | Val | TTG/CTG | Leu | Hb Johnstown |
| β116 | CAT | His | CAG/CAA | Gln | Hb Hafnia |
| β120 | AAA | Lys | AAT/AAC | Asn | Hb Riyadh |
| β132 | AAA | Lys | AAT/AAC | Asn | Hb Yamagata |
| β133 | GTG | Val | TTG/CTG | Leu | Hb Extremadura |
| β139 | AAT | Asn | AAG/AAA | Lys | Hb Hinsdale |
| β143 | CAC | His | CAG/CAA | Gln | Hb Little Rock |
| β145 | TAT | Tyr | TAG/TAA | Term. | Hb McKees Rock |

to elucidate the underlying nucleotide substitution in the original family A. Unfortunately, no members of this family could be reached for sampling, so DNA had to be extracted from an old hemolysate which was kept frozen in the laboratory as a potential reference sample for electrofocusing. DNA was successfully obtained from this sample and the other possible substitution corresponding to Hb Malmö, CAC→CAA (Fig. 1), turned out to be present in family A. Samples from both families were sequenced twice on two separate occasions, each using pooled material from two freshly performed separate amplifications.

The proband in family B was homozygous for a haplotype resembling haplotype I or V, both haplotypes being compatible with the findings in the sole representative of family A that could be investigated. In family B, the variant was present only in the proband and not in his mother or sister. The deceased father, a non-smoker, was treated for hypertension detected at the age of 38 and had a myocardial infarct at the age of 43. Three months later, he was hospitalized for treatment of transitory brain stem ischemia and had a hemoglobin concentration of 149 g/L and hematocrit of 42%. He died at the age of 49, presumably of a myocardial infarct. No other relatives of the father were available for investigation.

Looking through the other β-globin variants reported to the International Hemoglobin Information Center [10] revealed that 34 of the 316 β-globin variants assumed to be due to single amino acid substitutions could be caused by more than one type of point mutation at the DNA level (Table I). With the exception Hb Malmö, we only have experience with two of these variants. Hb Okayama has been found in Sweden in two seemingly unrelated patients, both of them showing the codon 2 CAC→CAA mutation. One of the carriers was an ethnic Swede while the other had relations to Austria and Germany. In addition, Hb Tacoma due to the codon 30 AGG→AGT substitution has been found in Sweden [6] in several members

**TABLE II. β- and α-Globin Variants Where Reported Amino Acid Substitutions Are Not Compatible With Single Point Nucleotide Substitutions**

| Variant | Position | Codon | Amino acid replacement | Assumed codon in variant | Reference |
|---|---|---|---|---|---|
| Hb Edmonton | β50 | ACT | Thr→Lys | AAA; AAG | [15] |
| Hb Bristol | β67 | GTG | Val→Asp | GAT; GAC | [16] |
| Hb Beckman | β135 | GCG | Ala→Glu | GAA; GAG | [17] |
| Hb J-Kurosh | α19 | GCG | Ala→Asp | GAT; GAC | [18] |

of three not obviously related families. However, all these families are of Finnish descent, so they are likely to share a common origin with other published cases carrying this variant [11].

The study of the list of reported β-globin variants also indicated that three of the β-globin and one of the α-globin variants reported were not compatible with single transitions or transversions in the normal globin gene sequence (Table II).

## DISCUSSION

It is well known that the same hemoglobin variant may occur independently in families of widely different ethnic origin. In Sweden this has been exemplified by the finding of Hb K-Ibadan and Hb Fukuyama in ethnic Swedes [5]. Sometimes de novo mutations are observed, most commonly when the mutations result in an unstable β-globin variant, e.g., Hb Köln [12] or Hb Altdorf [13].

That different nucleotide substitutions might cause the same hemoglobin variant was recently demonstrated by Molchanova et al. [14] who described two different mutations causing Hb G-Philadelphia. These mutations occur on either the normal α2-globin gene or on the hybrid α2α1-gene found on -$\alpha^{3.7}$ chromosomes. We now report the first example of a β-globin variant being caused by two different mutations. Furthermore, the two mutations at codon 97, CAC→CAA and CAC→CAG, both presenting as Hb Malmö, occurred in the same ethnic group. This finding reminds us that the sole fact that a hemoglobin variant is found in two families in the same population does not entitle us to regard the families as related.

For those hemoglobin variants, of which already more than 30 are described, where more than one underlying mutation is possible, reports containing not only the amino acid substitution but also the underlying mutation are desired. As exemplified in the present report, even hemolyzed red cells that have been stored frozen for many years can be used as the source of DNA for amplifications. Hence information about nucleotide substitutions could be obtained on several variants already described.

The finding in the literature of three β- and one α-globin variant that are not compatible with single point nucleotide substitutions is intriguing. The possible reasons for this discrepancy are, however, beyond the scope of this report.

## ACKNOWLEDGMENTS

The skillful technical assistance of Ms. Kerstin Fridh is gratefully acknowledged. This work was supported by a grant from the Medical Faculty, University of Lund.

## REFERENCES

1. Lorkin PA, Lehmann H, Fairbanks VF, Berglund S, Leonhardt T: Two new pathological haemoglobins: Olmsted β141 (H19) Leu→Arg and Malmö β97 (FG4) His→Gln. Biochem J 119:68P, 1970.
2. Fairbanks VF, Maldonado JE, Charache S, Boyer SH: IV: Familial erythocytosis due to electrophoretically undetectable hemoglobin with impaired oxygen dissociation (Hemoglobin Malmö, $\alpha_2\beta_2^{97\ gln}$). Mayo Clinic Proc 46:721–727, 1971.
3. Girino M, Riccardi A, Mosca A, Paleari R, Bonomo P: Double heterozygosity for hemoglobin Malmö [β97 (FG4) His→Gln] and β-thalassemia traits. Haematologica 74:187–190, 1989.
4. Landin B: Hb Karlskoga or $\alpha_2\beta_2$21(B3)Asp→His: a new slow-moving variant found in Sweden. Hemoglobin 17:201–208, 1993.
5. Landin B, Jeppsson J-O: Rare β chain hemoglobin variants found in Swedish patients during $HbA_{1c}$ analysis. Hemoglobin 17:303–318, 1993.
6. Jeppsson JO, Berglund S: Thin-layer isoelectric focusing for haemoglobin screening and its application to haemoglobin Malmö. Clin Chim Acta 40:153–158, 1972.
7. Higuchi R: Rapid, efficient DNA extraction for PCR from cells or blood. Amplifications 2:1–3, 1989.
8. Landin B, Rudolpi O, Ek B: Initiation codon mutation (ATG→ATA) of the β-globin gene causing β-thalassemia in a Swedish family. Am J Hematol 48:158–162, 1995.
9. Landin B, Berglund S, Lindhoff B: Hb Trollhättan [β20(B2)Val→Glu]—a new haemoglobin variant with increased oxygen affinity causing erythrocytosis. Eur J Haematol 53:21–25, 1994.
10. International Hemoglobin Information Center: IHIC variants list. Hemoglobin 18:77–161, 1994.
11. Deacon-Smith RA, Lee-Potter JP: An unstable haemoglobin, Hb Tacoma β30(B12) arg→ser, detected at birth by the demonstration of red cell inclusions. J Clin Pathol 31:883–887, 1978.
12. Landin B, Fröstad B, Brune M, Ljung R: Haemoglobin Köln as de novo mutations in Sweden: diagnosis by PCR and specific enzymatic cleavage. Eur J Haematol 52:156–161, 1994.
13. Landin B, Åström M: Unstable haemoglobin causing haemolytic anaemia: de novo mutation in Sweden identified by PCR. J Int Med 233:299–302, 1993.
14. Molchanova TP, Pobedimskaya DD, Ye Z, Huisman THJ: Two different mutations in codon 68 are observed in Hb G-Philadelphia heterozygotes. Am J Hematol 45:345–346, 1994.

15. Labossiere A, Hill JR, Vella F: A new βTp V hemoglobin variant: Hb Edmonton. Clin Biochem 4:114–117, 1971.
16. Steadman JH, Yates A, Huehns ER: Idiopathic heinz body anaemia: Hb-Bristol (β67(E11)Val→Asp). Br J Haematol 18:435–446, 1970.
17. Rahbar S, Lee T, Asmeron Y: Hb Beckman $\alpha_2\beta_2$135(H13)Ala→Glu: a new unstable variant and reduced oxygen affinity. Blood 78(Suppl 1):204a, 1991.
18. Rahbar S, Ala F, Akhavan E, Nowzari G, Shoa'i I, Zamanianpoor MH: Two new haemoglobins: Haemoglobin Perspolis [α64(E13)Asp→Tyr] and Haemoglobin J-Kurosh [α19(AB1)Ala→Asp]. Biochim Biophys Acta 427:119–125, 1976.